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Resource utilization of microalgae from biological soil crusts: biodiesel production associated with desertification control

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Abstract

With the continuing consumption of resources and increasingly prominent environmental issues, microalgal resource utilization has received extensive attention. In this study, based on the microalgal investigation in desert biological soil crusts (BSCs) using pyrosequencing technology, the cultivated crust microalgae were further isolated in order to obtain high quality microalgae for resource utilization. The results showed that with crust development and succession, microalgal diversity gradually decreased, including the number of operational taxonomic units (OTUs) and genus, although *Microcoleus* always was the dominant genera. Pyrosequencing obtained 630 OTUs of cyanobacteria, 25 OTUs of green algae and 9 OTUs of diatom; however, part of cultivated microalgae still could not yet be detected due to the DNA extraction preferences and errors caused by PCR amplification. After isolation, four strains were purified and cultivated, including two filamentous cyanobacteria *Microcoleus vaginatus* BSC-6 and *Scytonema javanicum* BSC-39, and two unicellular green algae *Chlorella* sp. BSC-24 and *Monoraphidium dybowskii* BSC-81. The two green algae grew fast (>250 mg L^{-1} d^{-1}), and achieved high lipid productivity up to 75-85 mg L^{-1} d^{-1}, with lipid content of 28.7-39.0%, thus was considered as promising feedstock for biodiesel production. In addition, the two crust cyanobacteria could be used to construct artificial cyanobacterial soil crusts in desertification control, although their biomass accumulation was not as high as that in the green algae. Ultimately, combining biodiesel production with desertification control would not only improve desert

environments, but also provide ideal places for the local microalgal resource exploitation, further promoting desert socioeconomic development.

Keywords: Desert; Biological soil crusts; Microalgae; Biodiesel; Cyanobacterial inoculation

1. Introduction

With the increasing depletion of non-renewable resources and prominent environmental issues, microalgal (for simplicity including cyanobacterial) resource utilization has recently received a great deal of attention [1,2]. Particularly, as an alternative important bioenergy feedstock, microalgae have been considered as a promising lipid source for biodiesel production [3]. At present, although some lipid-producing microalgal species have been studied, most of the microalgae come from culture collection libraries, such as the Culture Collection of the University of Texas [4], Freshwater Algae Culture Collection at the Institute of Hydrobiology [5], Microbial Culture Collection, National Institute for Environmental Studies [6], CSIRO Algal Culture Collection [7], and culture collection of algae of Göttingen University [8]. Lots of the lipid-producing microalgae in the culture collection libraries have undergone long-time moderate environments, and it is difficult to adapt well to the field changeable environmental conditions when they are cultivated on a large scale [9,10]. Therefore, it becomes an important issue to directly isolate excellent lipid-producing microalgae from harsh environments, so that the microalgae can adapt well to the cultivation environmental conditions.

In arid and semi-arid desert regions, the environments are generally characterized by a series of harsh conditions, such as poor soil, extreme drought, high salinity, pH and radiation, large temperature variation and accustomed wind and sand storm [11,12]. In such extreme evil environments, many types of organisms are restricted, while

biological soil crusts (BSCs) can be widely distributed there because of their unique physio-ecological characteristics, and even occupy more than 70% of the living coverage in some areas [13,14]. BSCs are the complex biological soil mosaic layers within the uppermost millimeters of the soil, generally first colonized by microalgae [15,16]. As the pioneer, microalgae not only play an irreplaceable role in crust formation, development and succession, but also have important ability to adapt to the field environmental conditions [16,17]. Therefore, isolating lipid-producing microalgae from desert BSCs may provide more high quality microalgal species for large scale cultivation.

Desertification has brought a series of threatens to the local environment and socio-economic development. Isolating lipid-producing microalgae in desert regions not only provides the possibility for biodiesel production to promote local economic development, some microalgal species could also be used to accelerate the development and succession of BSCs for desertification control [14,17]. Therefore, combining desertification control and biodiesel production together would further promote the socio-ecological development in desert regions. Generally, high lipid-producing microalga are eukaryotic, but at present most of the investigations on crust microalgae are still concentrated in prokaryotic cyanobacteria [15-17]; while there has been very little work investigating on crust eukaryotic microalgae [18,19]. A comprehensive study on the composition of crust microalgae is important because it will not only help us

understanding the development, succession and ecological functions of BSCs in deep, but also have great value in microalgal resource utilization in desert regions.

In this study, on the basis of comprehensive microalgal investigation in the different developmental and successional BSCs in the Shapotou region (the Tengger Desert), the cultivated crust microalgae were isolated and purified. Then from the point of view of microalgal lipid content, biodiesel production associated with artificial cyanobacterial soil crust construction, the potential of microalgal resources from BSCs were explored, and the results would provide significant guidance for the resource utilization in desert regions.

2. Materials and methods

2.1 Sampling

BSCs including cyanobacterial, lichen and moss soil crusts (Table 1) were sampled from the Shapotou region (a part of Ningxia Hui Autonomous Region), located at the southeast edge of the Tengger Desert (37°32′ N and 105°02′ E). The BSCs were collected into the sterilized Petri dishes with a sharp shovel to make sure the crust samples were in their natural thickness. The sampling was conducted randomly from the interspaces between shrubs (0.2 m away from the shrubs), and all the samples were carried to the laboratory as soon as possible for subsequent analysis. Each type of BSCs was sampled at three different sites as repetition.

2.2 Physicochemical characteristics

126 Crust thickness was measured using a Vernier caliper. Crust coverage of
127 cyanobacteria, lichens, mosses and dominant species were visually assessed and
128 identified under a microscope with charge-coupled device (CCD, LY-WN-SUPER HP
129 CCD, China) according to the description of Wu et al. [20]. Chlorophyll-a (Chl-a)
130 content was measured in the ethanol extract using a spectrophotometry [21], and
131 polysaccharides content was determined using the phenol-sulfuric acid method [19].

132 **2.3 Crust pyrosequencing data analysis**

133 Total DNA was extracted from the BSCs with Mag-Bind Soil DNA Kit (OMEGA,
134 USA) following the manufacturer's instruction, and 16S and 18S rRNA gene segments
135 were PCR amplicated from each sample DNA according to the method of Zhang et al.
136 [22]. The amplicons were used for pyrosequencing analysis on a Roche GS FLX
137 Titanium machine (Roche, USA), which was carried out by Majorbio Biotech Co. Ltd.
138 (Shanghai, China). All the sequences then were submitted to the NCBI database under
139 the accession numbers SRP063082 and SRP063545. The low quality sequences were
140 discarded and the trimmed sequences (primers and adaptors were removed) were
141 clustered into different operational taxonomic units (OTUs) at 97% similarity level. The
142 taxonomic annotation information of each OTU were then extracted from the SILVA
143 SSU rRNA database. Although the microbial community in the BSCs has been
144 analyzed at phylum level by Zhang et al. [22], microalgal composition is still unknown.
145 Therefore, in this study the same pyrosequencing data were used to analyze microalgal
146 composition in the BSCs from Shapotou region. According to the number of sequences

147 in each OTU, microalgal abundance in genera level was calculated and those
148 microalgae with more than 5% abundance were considered as the dominant.

149 **2.4 Crust microalgal isolation, indentification and cultivation**

150 For microalgal isolation, crust samples were inoculated on BG-11, BBM, HB-D1
151 and SE solid agar media, respectively, according to the previous description [15,16,23].
152 The inoculations were placed into an incubator for 15-20 d (25±1°C), illuminated with
153 cool white fluorescent light at 40-60 μE m^{-2} s^{-1}. Then microalgal single colonies with
154 good growth state were picked up under a stereomicroscope and purified into BG-11
155 liquid medium. When the purified microalgae accumulated to a certain biomass,
156 microalgal microscopic morphology was observed, 16S or 18S rDNA was sequenced
157 according to the methods of Moreora et al. [25] and He et al. [24]. All the sequences
158 have been submitted to the NCBI database with accession numbers MH412926,
159 MH412927, KX395732 and KX395736.Then the purified microalgae were further
160 cultivated with BG-11 liquid medium at their respective appropriate conditions. During
161 the cultivation process, microalgal dry weight was measured to evaluate the biomass
162 variation [24].

163 **2.5 Lipid producing properties of crust green algae**

164 After cultivation, two crust green algae were harvested and their lipids were
165 extracted using a Soxhlet reflux extractor with chloroform/methanol (2/1, v/v) [24]. The
166 extracted microalgal lipids were then esterified with methanol in acidic condition, and
167 the fatty acid methyl esters (FAMEs) were identified and quantified using a gas

chromatograph mass spectrometry (GC-MS; Thermo Scientific ITQ 700, USA) with a fused silica capillary column (Agilent Technologies, USA) and flame ionization detector (FID) [26]. Microalgal fatty acid compositions (%) were then calculated from the standard calibration curves of Supelco 37 component FAME mix (Sigma-Aldrich, USA), and microalgal lipid content, biomass and lipid productivity were calculated according to the methods of Zhou et al. [9] and Wu et al. [26].

2.6 Artificial cyanobacterial soil crust construction

The other two crust cyanobacteria were harvested and spray inoculated (at a ratio of 10:1) into the Petri-dishes containing shifting sand to construct artificial cyanobacterial soil crusts. The inoculated Petri-dishes were then placed in a greenhouse (25±1°C), illuminated with cool white fluorescent light at about 40 μE m^{-2} s^{-1}, and watered everyday with 10 mm distilled water. During the experiment, the biomass of inocula (Chl-*a* content) was measured according to the description of Lan et al. [21].

3. Results and discussion

3.1 Microalgal composition in BSCs

The BSCs in our experimental regions mainly include cyanobacterial, lichen and moss soil crusts, and average 20471 and 21391 reads per sample have been obtained for prokaryotic and eukaryotic microbes, respectively [22]. Based on the pyrosequencing data, the OTUs for prokaryotic cyanobacteria and eukaryotic green algae and diatom were drawn out from the original crust microbial communities. The results showed that

189 with the development and succession from cyanobacterial to lichen and moss soil crusts,
190 crust photosynthetic biomass gradually increased (indicated by Chl-*a* content; Table1),
191 while microalgal diversity decreased, including the number of OTUs and genus (Table
192 2), although *Microcoleus* always was the dominant genera (Table 3). That the decrease
193 of microalgal diversity in lichen and moss soil crusts may be due to the living space
194 being occupied by a large number of lichens and mosses, because it is very clear that
195 lichen and moss biomass increases gradually with crust development and succession
196 [14,16].

197 Although some microalgal compositions in BSCs have been reported, the most
198 investigations are still concentrated in prokaryotic cyanobacteria [17,19]. The sporadic
199 investigations on crust eukaryotic microalgae have found that some species in
200 *Chlorophyta* and *Bacillariophyta* are the main crust eukaryotic microalgae [18,19]. For
201 example, Bhatnagar reported four species of crust green algae in the Thar Desert of
202 Indian [18], and Wang et al. found three species of crust green algae and diatom
203 respectively in the Qubqi Desert of China [19]. However, all those investigations are
204 based on microalgal morphological observation after cultivation, thus lots of crust
205 microalgal information may be lost due to the selectivity of media. Therefore, in the
206 present study, it was expected to obtain much more microalgal information through
207 crust total DNA extraction, 16S and 18S rDNA amplification and pyrosequencing. As
208 the results, although as many as 664 OTUs of microalgae were obtained, including 25
209 genus of cyanobacteria, 13 genus of green algae and 5 genus of diatom (Table 2), some

cultivated microalgae, such as the species in the genus *Chlorella* and *Monoraphidium*, still could not be detected yet. That might be because the DNA extraction process preferred some species, and PCR amplification also could cause errors due to the catalytic efficiency variation [27].

3.2 Microalgal indentification and cultivation

After microalgal isolation, those with good growth state were chosen for further resource utilization, including microalgae BSC-06, BSC-24, BSC-39 and BSC-81. Both BSC-06 and BSC-39 are filamentous cyanobacteria, the former is unbranched filaments, without heterocyst; while the later has false branches and heterocysts (Fig. 1 A and B). Therefore, BSC-06 and BSC39 were temporarily nominated as *Microcoleus* like BSC-06 and *Scytonema* like BSC-39. After the 16S rDNA sequence phylogenetic analysis, the two crust cyanobacteria were identified as *M. vaginatus* BSC-06 and *S. javanicum* BSC-39 (Fig. 2A). BSC-24 and BSC-81 are unicellular green algae, and were suspected as some species in the genus *Chlorella* and *Monoraphidium* according to their microscopic morphology (Fig. 1C and D). From the 18S rDNA sequence phylogenetic analysis, the two crust green algae were finally identified as *Chlorella* sp. BSC-24 and *M. dybowskii* BSC-81 (Fig. 2B).

To harvest microalgal biomass is an important link to resource utilization, and sufficient biomass would be the great guarantee for microalgal resource utilization [10,26]. Therefore, the four isolated crust microalgae were further cultivated to determin their biomass accumulation. After cultivation, microalgal biomass increased

gradually, and at the end of experiment the two crust cyanobacterial biomass increased by 4.4 and 3.8 folds, respectively (for *M. vaginatus* BSC-6 and *S. javanicum* BSC-39; Fig. 3A). Whereas, during the similar cultivation period, the two crust green algae *Chlorella* sp. BSC-24 and *M. dybowskii* BSC-81 increased 17.0 and 24.7 folds (Fig. 3B). Through microalgal cultivation, it was found that the biomass accumulation in the two crust green algae was much more than that in the two crust cyanobacteria. Ultimately, the biomass productivity reached 262 and 218 mg L^{-1} d^{-1} for the two green algae *Chlorella* sp. BSC-24 and *M. dybowskii* BSC-81, while only 53 and 40 mg L^{-1} d^{-1} for the two cyanobacteria *M. vaginatus* BSC-6 and *S. javanicum* BSC-39.

The growth difference between the cyanobacteria and green algae on the one hand may be due to their respective evolutionary positions [28], and relatively higher evolution of green algae may be more willing to accumulate high biomass to achieve the purpose of self-reproduction. On the other hand the different growth capability may also be related to their morphological difference. Because compared with the unicellular green algae in the present study, cyanobacterial filaments are easier to clump together, so that the internal filaments are not readily supplied with available nutrients, light and other growing conditions.

3.3 Microalgal lipid-producing properties

Lipids can be synthesized and accumulated in diverse microalgae, however cyanobacteria can only produce low quantity of lipids [1], and thus the current investigations on lipid-producing microalgae are mainly launched in green algae and

252 diatoms, such as some species in the genus *Scenedesmus* and *Phaeodactylum* [29,30].
253 In the present study, lipid contents in the two crust green algae were further measured,
254 and it was found the values were as high as 28.7% and 39.0% for *Chlorella* sp. BSC-24
255 and *M. dybowskii* BSC-81, respectively (Fig. 4). Considering the biomass accumulation,
256 ultimately the two crust green algae achieved a lipid productivity of 75-85mg L^{-1} d^{-1}
257 (Table 4).
258 Microalgae produce lipids through synthesizing fatty acids as building blocks,
259 therefore the fatty acid composition is also a significant determining factor for
260 microalgal lipid production [1,26,29]. In the present study, it was found the fatty acid
261 compositions of two crust green algae were mainly concentrated between C16-C18 (>
262 96%), especially the fatty acids C16: 0 and C18: 1 accounted for more than 60% of the
263 total fatty acids (Fig. 5). In both green algae, fatty acids were either saturated or
264 unsaturated, and the unsaturated fatty acids contained one or more double bonds on
265 their carbon chains. From the fatty acid profiles, it was found polyunsaturated fatty
266 acids (PUFAs) were mainly concentrated in C18:2, C18:3 and C18:4 (Fig. 5).
267 Comparing the lipid productivities, it was found the two isolated crust green algae
268 produced higher lipids than the most reported microalgae [9,29,31]. In detail, the
269 biomass productivity, lipid content and productivity of the two crust green algae were
270 compared with the results from other 30 microalgal strains reported by Rodolfi et al.
271 [31] (Table 4). The results showed that although some microalgal strains obtained
272 higher biomass productivity, such as *Porphyridium cruentum* (366.3 mg L^{-1} d^{-1}) and

Tetraselmis suecica F&M-M33 (317.6 mg L^{-1} d^{-1}), the two crust green algae achieved higher lipid productivity. In the report of Feng et al. [32], although the higher lipid productivity was obtained in *Chlorococcum pamirum* through NaCl induction, adding NaCl would increase the cultivation cost. At the same time, their results also indicate that crust green algae *Chlorella* sp. BSC-24 and *M. dybowskii* BSC-81 would accumulate more lipids through the induction of NaCl or other conditions. Because it has been confirmed that microalgal lipid content would increase in the conditions of nutritional deficiencies or other physical and chemical stresses [1,29,30,32].

3.4 Biodiesel production associated with desertification control

Biodiesel production is an important direction for microalgal resource utilization. Especially with the increasing depletion of fossil energy, microalgae are regarded as the promising feedstock of future for sustainable biodiesel production [3,9], because microalgae have high photosynthetic efficiency and growth rate, can be cultivated on non-arable lands, and effectively convert CO_2 into high energy density triacylglycerol (TAG) [2,29,33]. In the present study, the lipids produced by crust green algae *Chlorella* sp. BSC-24 and *M. dybowskii* BSC-81 fully met the requirement of biodiesel production [33,34]. In addition, the quality parameters of biodiesel produced by the two crust green algae, including cetane number (CN) and iodine value (IV), were also predicted according to the description of Xia et al. [29] and He et al. [24]. CN is widely used to indicate the ignition delay time and combustion quality, the higher the CN is, the better the ignition property is [34]. The CN for biodiesel should be at a minimum of

294 51 according to the European standard UNE-EN 14214. Meanwhile the UNE-EN
295 14214 also standardizes the maximum of 120 g I_2 100 g^{-1} for IV, and the higher IV
296 would result in the polymerization of glycerides, forming the deposits and ultimately
297 deteriorating the lubricating oil [26,29]. In the present study, the calculated CN and IV
298 for the two crust green algae were in line with UNE-EN 14214 standard. CN values
299 were 54.83 and 56.39; while IV values were 102.48 and 85.07 g I_2 $100g^{-1}$ for *Chlorella*
300 sp. BSC-24 and *M. dybowskii* BSC-81, respectively.
301 Microalgal cultivation place is not only directly related to the cultivation cost, but
302 also reflects the rationality of land use. Therefore, desert lands are proposed as the ideal
303 microalgal cultivation place due to the abundant light resource and lower land cost
304 [10,35]. In desert regions, environment and economy are two prominent problems
305 hinder the local social development. However, cultivating lipid-producing microalgae
306 for biodiesel production can not only promote desert economic development, but some
307 species also can be used to construct BSCs in the process of desertification control
308 [15,19], such as crust cyanobacteria *M. vaginatus* BSC-6 and *S. javanicum* BSC-39
309 isolated in this study. That is because although compared with the high lipid-producing
310 green algae, the two cyanobacteria accumulated the lower lipid content [1] and biomass
311 (Fig. 3), these filamentous cyanobacteria were able to secrete large amounts of
312 extracellular polysaccharides, which has a strong cementing capacity [17]. Therefore,
313 combining biodiesel production and desertification control will further promote desert
314 socio-economic development.

315 After cyanobacterial inoculation on the sand, the cyanobacterial filaments would
316 be contact with sand particles in direct. When the filaments grew and moved, they
317 would inevitably entangle with sand particles, forming the aggregates of cyanobacteria
318 and sand particles. At the same time, the secreted extracellular polysaccharides
319 gradually accumulated in association with cyanobacterial growth, further conglutinating
320 additional sand particles to form firmer and stable crust structure, so as to achieve the
321 target of sand fixation [11,14]. In the present study, the inoculated cyanobacteria grew
322 quickly due to the watering every day, and reached 139.3 mg Chl-*a* m^{-2} after a month,
323 increasing by 7.8 folds compared with the biomass at beginning (Fig. 6). However, in
324 the practice of constructing artificial cyanobacterial soil crusts, water is an important
325 factor affecting crust formation and development, since water is very limited in desert
326 regions. To ensure adequate water is an important prerequisite for crust formation and
327 development after cyanobacterial inoculation [15,19]. Although large amounts of
328 watering can ensure the survival rate of inoculated cyanobacteria, it will greatly
329 increase the project cost, as well as result in unnecessary waste of water resource.
330 Therefore, proper watering after cyanobacterial inoculation can not only ensure crust
331 growth, but also reduce the maintenance cost. If crust construction and lipid-production
332 are combined together, the waste cultivation liquid after harvesting lipid-producing
333 microalgae can also be used as water resource, as well nutrients, to promote crust
334 growth. On the other hand, at the same time of constructing artificial cyanobacterial soil
335 crusts for desertification control, the desert lands in return can be used for free to

cultivate lipid-producing microalgae. That will further reduce the land cost in biodiesel production, because it has been reported that in some cases the land cost for microalgal biodiesel production can occupy as much as 11.3% of the total capital cost [36].

4. Conclusions

In this study, the microalgal composition of biological soil crusts (BSCs) was investigated by pyrosequencing. Then, two cyanoacteria *Microcoleus vaginatus* BSC-6 and *Scytonema javanicum* BSC-39, and two green algae *Chlorella* sp. BSC-24 and *Monoraphidium dybowskii* BSC-81 were further isolated from the BSCs. The two crust green algae achieved higher biomass productivity than cyanobacteria, with high lipid content and productivity, thus were regarded as the promising feedstock for biodiesel production. The two crust cyanobacteria also could be used to construct artificial cyanobacterial soil crusts in desertification control, which would not only provide the free desert lands for lipid-producing microalgal cultivation and biodiesel production, but also promote the reuse of waste water after lipid-producing microalgal cultivation. Together, biodiesel production associated with desertification control would promote desert socio-economic development, and our results imply the desert BSCs are the important resource for microalgal utilization.

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462 **Table 1.** Physicochemical characteristics of different successional biological soil crusts

	Cyanobacterial soil crusts	Lichen soil crusts	Moss soil crusts
Thickness (mm)	3.80 ± 0.81 a*	8.10 ± 1.72 b	16.24 ± 2.87 c
Cyanobacterial coverage (%)	>95	<20	0
Lichen coverage (%)	0	>70	0
Moss coverage (%)	<5	<10	100
Dominant species	*Microcoleus vaginatus*	*Collema* sp.	*Bryum* sp.
Chl-*a* content (μg cm^{-2})	2.83 ± 0.20 a	6.18 ± 1.11 b	16.20 ± 2.09 c
Polysaccharides content (μg cm^{-2})	42.55 ± 16.54 a	84.17 ± 6.77 b	478.84 ± 30.74 c

463 * For a given crust parameter, values with different letters are significantly different at 0.05 level

464 (P<0.05).

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Table 2. Microalgal diversity and the dominant genus in the different successional biological soil crusts.

	Cyanobacterial soil crusts	Lichen soil crusts	Moss soil crusts
Number of cyanobacterial OTUs	630	235	87
Number of cyanobacterial genus	25	16	13
Dominant cyanobacterial genus	*Crinalium, Microcoleus, Oscillatoria, Phormidium, Symploca*	*Microcoleus, Nostoc*	*Calothrix, Crinalium, Microcoleus, Nostoc, Symploca, Tolypothrix*
Number of green algal OTUs	25	10	7
Number of green algal genus	13	6	5
Dominant green algal genus	*Chlorosarcinopsis, Enallax*	*Chloromonas, Chlorosarcinopsis, Enallax, Prasinoderma, Pyramimonas*	*Gungnir, Hafniomonas, Lobosphaera, Neochlorosarcina, Pyramimonas*
Number of diatom OTUs	9	4	1
Number of diatom genus	5	3	1
Dominant diatom genus	*Campylodiscus*	*Campylodiscus*	*Nitzschia*

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492 **Table 3.** Microalgal community compositions (genera level) in the different

493 successional biological soil crusts (+++ dominant genus).

	Cyanobacterial soil crusts	Lichen soil crusts	Moss soil crusts
Cyanobacteria			
Anabaena	+		
Arthronema	+	+	
Calothrix	+	+	+++
Chlorogloeopsis	+	+	
Chroococcidiopsis	+		
Crinalium	+++	+	+++
Cyanobium	+		+
Cyanothece	+	+	+
Dolichospermum	+		
Fischerella	+	+	+
Gloeothece	+		+
Hapalosiphon		+	
Leptolyngbya	+		
Lyngbya	+	+	
Microcoleus	+++	+++	+++
Nodularia	+		
Nostoc	+	+++	+++
Oscillatoria	+++	+	+
Phormidium	+++	+	+
Planktothricoides	+		
Planktothrix	+		
Scytonema	+	+	+
Stigonema	+	+	
Symploca	+++	+	+++
Synechococcus	+		
Tolypothrix	+	+	+++
Unclassified cyanobacteria	+	+	+
Green algae			
Acrosiphonia	+		
Cephalomonas	+		
Chlamydomonas	+		
Chloromonas	+	+++	
Chlorosarcinopsis	+++	+++	
Dactylococcus	+		
Enallax	+++	+++	
Gungnir			+++
Hafniomonas			+++
Halosphaera		+	
Hemiflagellochloris	+		
Lobosphaera	+		+++
Mantoniella	+		
Neochlorosarcina			+++
Prasinoderma	+	+++	
Pyramimonas	+	+++	+++
Tabris	+		
Unclassified green algae		+	+
Diatom			
Campylodiscus	+++	+++	
Cymbella	+		
Melosira		+	
Navicula	+		
Nitzschia		+	+++
Pseudohimantidium	+		
Thalassiothri	+		

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496 **Table 4.** Lipid content and productivities of different microalgae species.

	Biomass productivity (mg L-1 d-1)	Lipid content (%)	Lipid productivity (mg L-1 d-1)
Chlorella sp. BSC-24*	261.7	28.7	75.1
Monoraphidium dybowskii BSC-81*	217.9	39.0	85.1
Porphyridium cruentum	366.3	9.5	34.8
Tetraselmis suecica F&M-M33	317.6	8.5	27.0
Tetraselmis sp. F&M-M34	295.2	14.7	43.4
Tetraselmis suecica F&M-M35	282.2	12.9	36.4
Phaeodactylum tricornutum F&M-M40	239.6	18.7	44.8
Nannochloropsis sp. F&M-M26	206.1	29.6	61.0
Nannochloropsis sp. F&M-M27	197.5	24.4	48.2
Nannochloropsis sp. F&M-M24	177.3	30.9	54.8
Nannochloropsis sp. F&M-M29	174.1	21.6	37.6
Ellipsoidion sp. F&M-M31	172.6	27.4	47.3
Nannochloropsis sp. F&M-M28	170.6	35.7	60.9
Nannochloropsis CS 246	170.2	29.2	49.7
Isochrysis sp. (T-ISO) CS 177	168.3	22.4	37.7
Pavlova salina CS 49	159.9	30.9	49.4
Pavlova lutheri CS 182	141.4	35.5	50.2
Isochrysis sp. F&M-M37	138.0	27.4	37.8
Skeletonema sp. CS 252	85.8	31.8	27.3
Thalassiosira pseudonana CS 173	84.5	20.6	17.4
Skeletonema costatum CS 181	82.5	21.1	17.4
Chaetoceros muelleri F&M-M43	64.9	33.6	21.8
Chaetoceros calcitrans CS 178	44.2	39.8	17.6
Chlorococcum sp. UMACC 112	278.2	19.3	53.7
Scenedesmus sp. DM	255.5	21.1	53.9
Chlorella sorokiniana IAM-212	231.6	19.3	44.7
Chlorella sp. F&M-M48	225.1	18.7	42.1
Scenedesmus sp. F&M-M19	208.2	19.6	40.8
Chlorella vulgaris F&M-M49	200.5	18.4	36.9
Scenedesmus quadricauda	190.8	18.4	35.1
Monodus subterraneus UTEX 151	188.8	16.1	30.4
Chlorella vulgaris CCAP 211/11b	169.8	19.2	32.6

497 * *Chlorella* sp. BSC-24 and *Monoraphidium dybowskii* BSC-81 are isolated in our study, and other

498 microalgal strains are drawn from the report of Rodolfi et al. [31].

Figure captions:

Fig. 1. The common cultivated crust microalgae including *Microcoleus* like BSC-6 (A), *Scytonema* like BSC-39 (B), *Collema* like BSC-24 (C) and *Monoraphidium* like BSC-81 (D).

Fig. 2. Maximum-likelihood tree of the cultivated crust cyanobacteria (A) and green algae (B) based on 16S and 18S rDNA sequences respectively. BSC-x indicates the microalgae cultured in our experiment, and the text in brackets shows the NCBI accession numbers of the different microalgal species.

Fig. 3. Growth curves of the cultivated crust cyanobacteria (A) and green algae (B).

Fig. 4. Lipid content, biomass and lipid productivity of the two crust green algae *Chlorella* sp. BSC-24 (A) and *Monoraphidium dybowskii* BSC-81 (B).

Fig. 5. Fatty acid compositions (%) of the two crust green algae *Chlorella* sp. BSC-24 (A) and *Monoraphidium dybowskii* BSC-81 (B).

Fig. 6. Growth curves of the inoculated cyanobacteria (*Microcoleus vaginatus* BSC-6 and *Scytonema javanicum* BSC-39) on shifting sand.

Fig. 1.

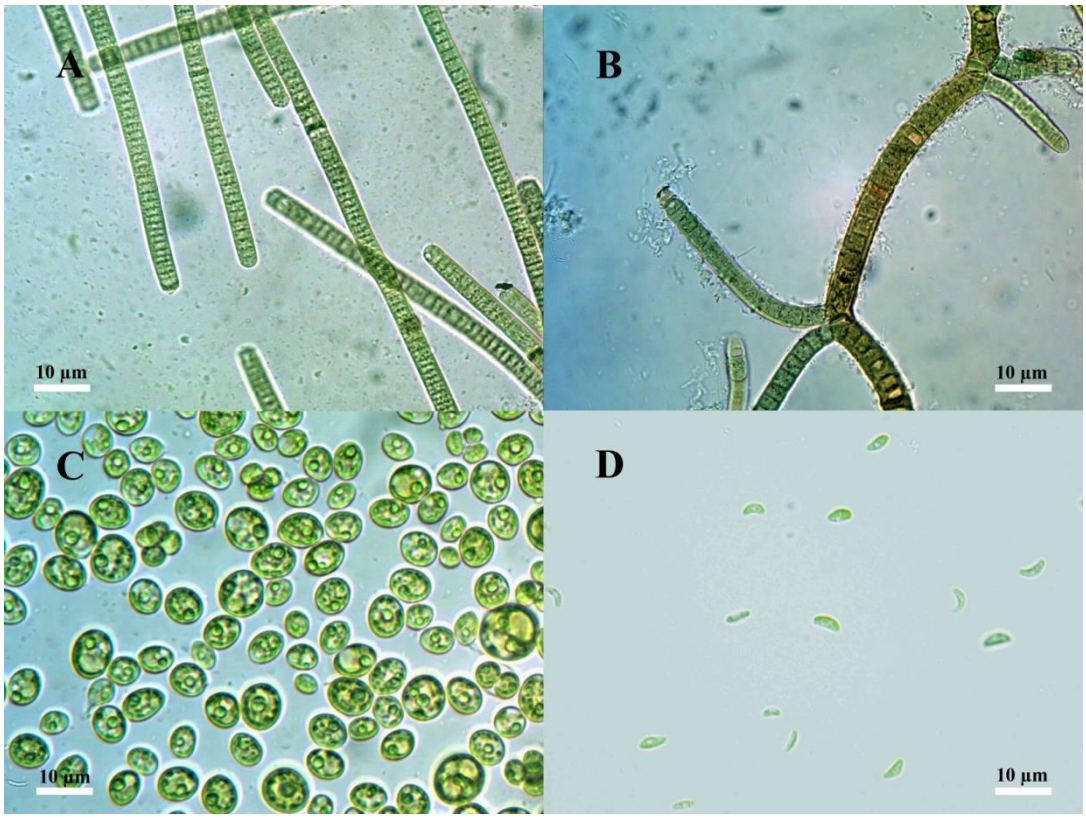


Fig. 2.

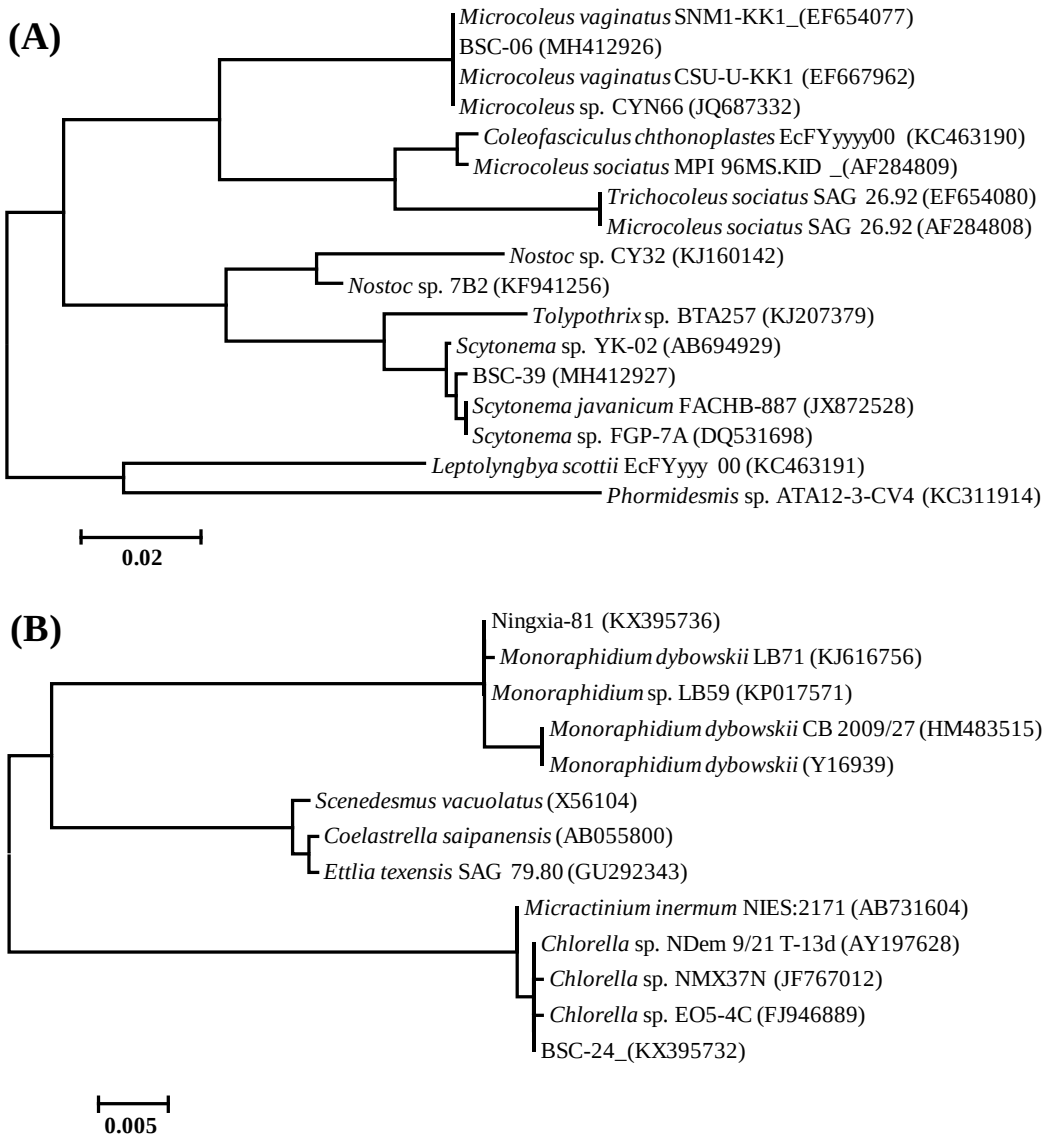
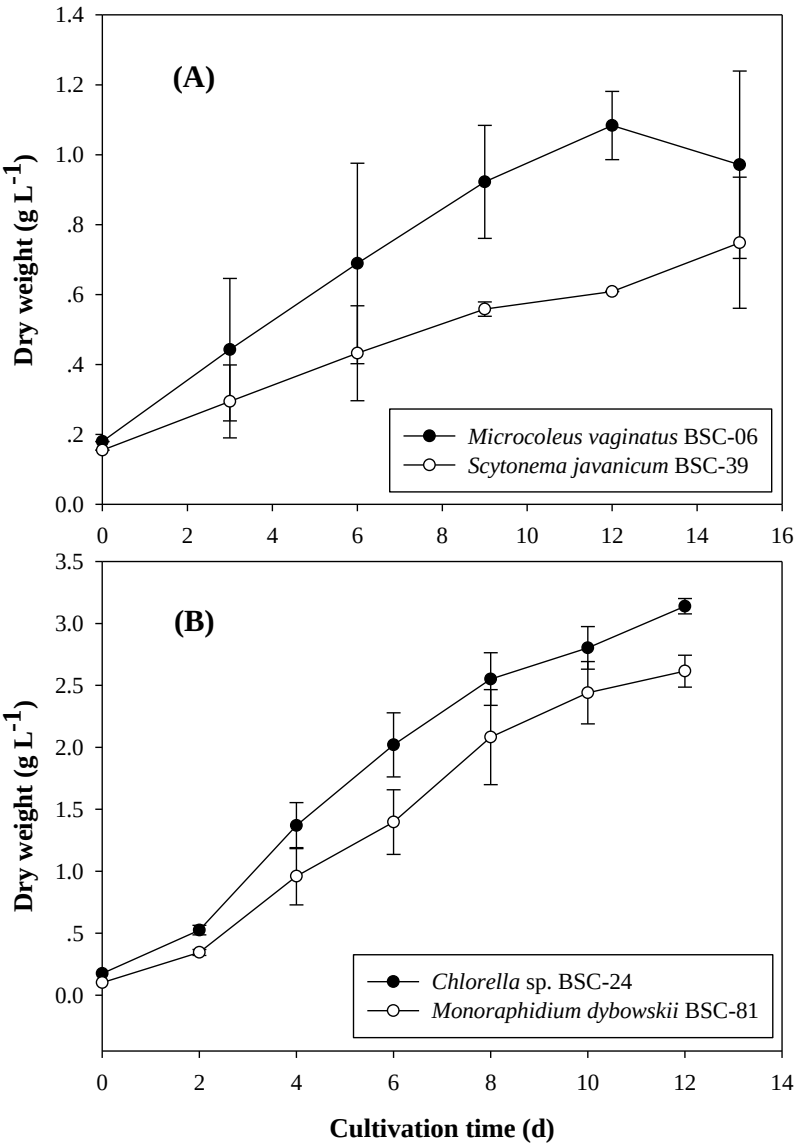

(A)
Microcoleus vaginatus SNM1-KK1_(EF654077)
BSC-06 (MH412926)
Microcoleus vaginatus CSU-U-KK1 (EF667962)
Microcoleus sp. CYN66 (JQ687332)
Coleofasciculus chthonoplastes EcFYyyyy00 (KC463190)
Microcoleus sociatus MPI 96MS.KID _(AF284809)
Trichocoleus sociatus SAG 26.92 (EF654080)
Microcoleus sociatus SAG 26.92 (AF284808)
Nostoc sp. CY32 (KJ160142)
Nostoc sp. 7B2 (KF941256)
Tolypothrix sp. BTA257 (KJ207379)
Scytonema sp. YK-02 (AB694929)
BSC-39 (MH412927)
Scytonema javanicum FACHB-887 (JX872528)
Scytonema sp. FGP-7A (DQ531698)
Leptolyngbya scottii EcFYyyy 00 (KC463191)
Phormidesmis sp. ATA12-3-CV4 (KC311914)
0.02
(B)
Ningxia-81 (KX395736)
Monoraphidium dybowskii LB71 (KJ616756)
Monoraphidium sp. LB59 (KP017571)
Monoraphidium dybowskii CB 2009/27 (HM483515)
Monoraphidium dybowskii (Y16939)
Scenedesmus vacuolatus (X56104)
Coelastrella saipanensis (AB055800)
Ettlia texensis SAG 79.80 (GU292343)
Micractinium inermum NIES:2171 (AB731604)
Chlorella sp. NDem 9/21 T-13d (AY197628)
Chlorella sp. NMX37N (JF767012)
Chlorella sp. EO5-4C (FJ946889)
BSC-24_(KX395732)
0.005

Fig. 3.


(A)
Dry weight (g L-1)
Microcoleus vaginatus BSC-06
Scytonema javanicum BSC-39
(B)
Dry weight (g L-1)
Chlorella sp. BSC-24
Monoraphidium dybowskii BSC-81
Cultivation time (d)

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549 **Fig. 4.**

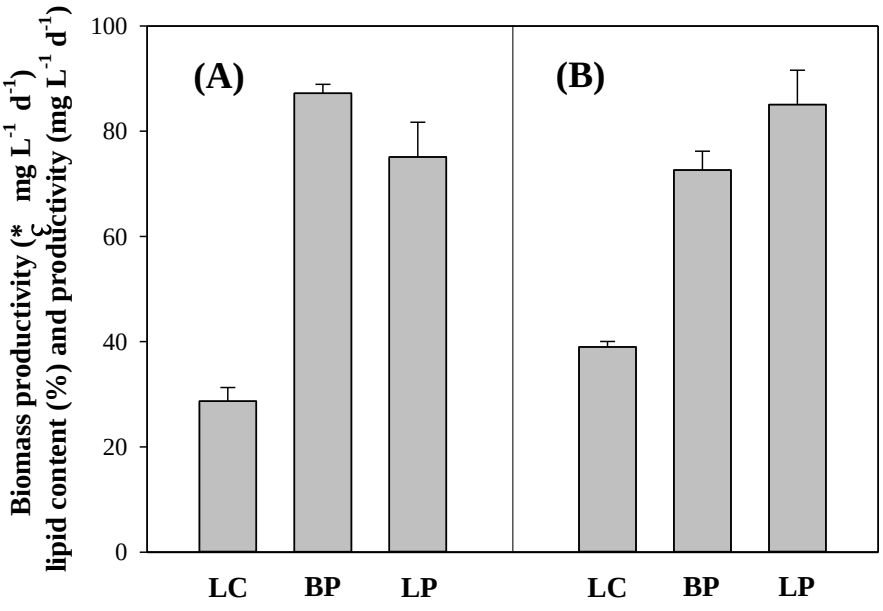

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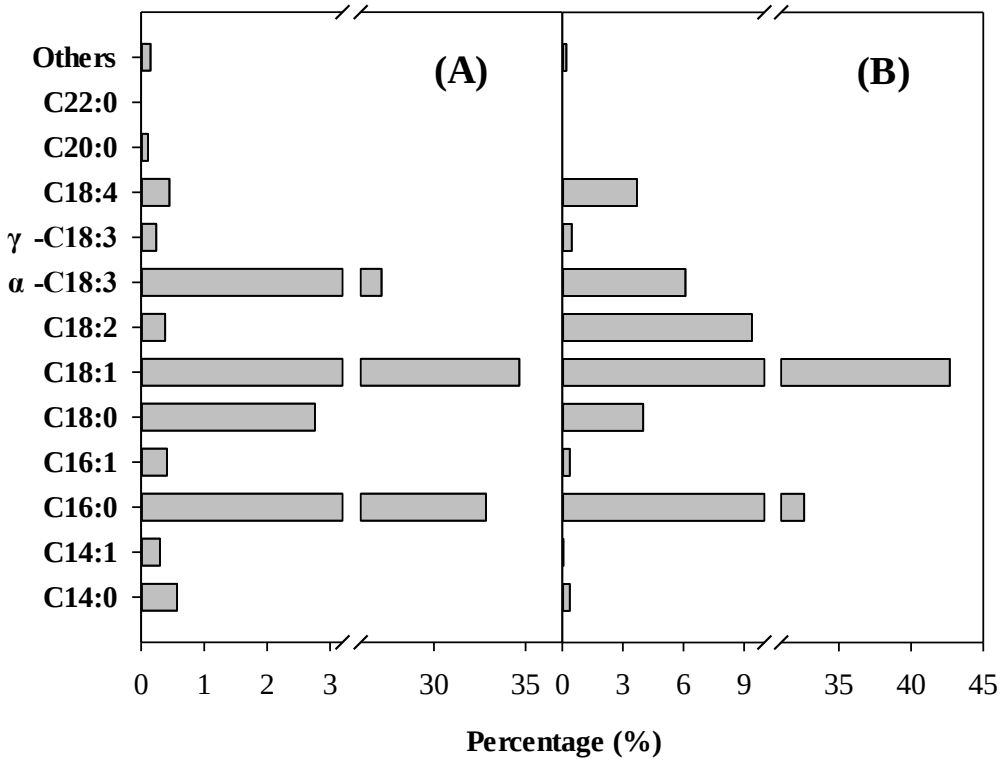
Fig. 5.


(A)
(B)
Others
C22:0
C20:0
C18:4
γ -C18:3
α -C18:3
C18:2
C18:1
C18:0
C16:1
C16:0
C14:1
C14:0
0 1 2 3 30 35
0 3 6 9 35 40 45
Percentage (%)

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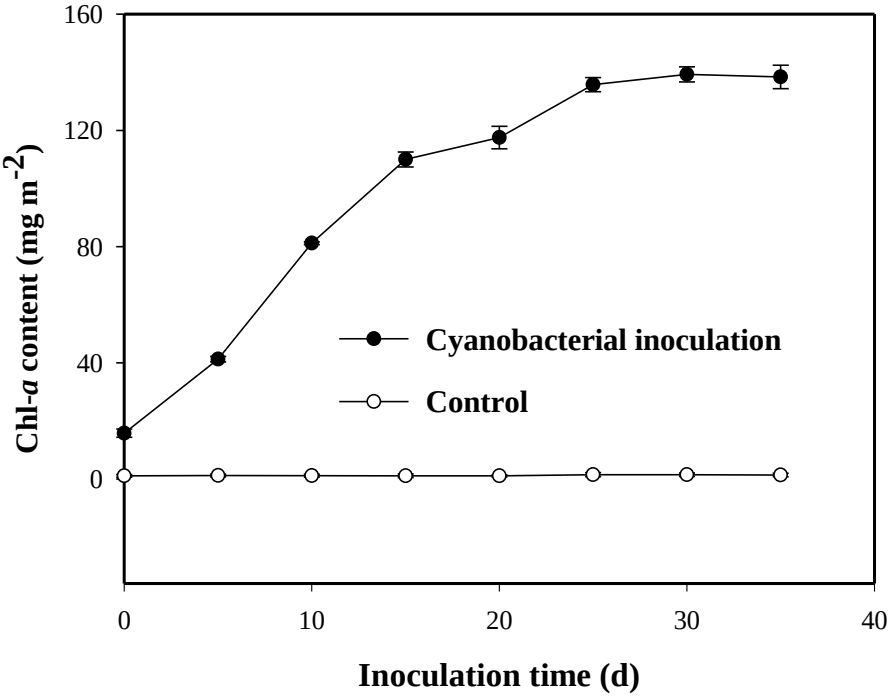
577 **Fig. 6.**



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